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Form PTO-1390 P21772.P01	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER P21772
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 1.5) 09/926718

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR00/01302	15 May 2000	08 June 1999

TITLE OF INVENTION
METHOD FOR OBTAINING FROM A CULTURE MEDIUM OF MICROALGAE, A HEAT-STABLE EXTRACT WITH ANTIOXIDANT WOUND HEALING ACTIVITY

APPLICANT(S) FOR DO/EO/US

Jacques POINT, Jean-CLaude BACCOU, and Bruno BAROUX

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. X This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. X This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
4. X The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. X A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. X is attached hereto (required only if not communicated by the International Bureau).
   b. X has been communicated by the International Bureau.
   c. is not required, as the application was filed in the United States Receiving Office (RO/US).
6. An English language translation of the International Application as filed (35 U.S.C. 371 (c)(2)).
7. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. are attached hereto (required only if not communicated by the International Bureau).
   b. have been communicated by the International Bureau.
   c. have not been made; however, the time limit for making such amendments has NOT expired.
   d. have not been made and will not be made.
8. An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3))
9. X An oath or declaration of the inventor(s) (35 U.S.C. .371(c)(4)).
   "Unexecuted"
10. An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (U.S.C. 371(c)(5)).

Items 11 to 16 below concern other document(s) or information included:

11. Assignee: AQUAMER(SAEM) of Méze, FRANCE
12. An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
13. An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
14. A FIRST preliminary amendment.
    A SECOND or SUBSEQUENT preliminary amendment.
15. A substitute specification.
16. A change of power of attorney and/or address letter.
17. Figure of Drawing to be published_____
18. X Other items or information:
    Cover Sheet and International Application as published in French
    PCT/IB/304(in French).
    PCT/IB/308(in French).
    PCT/ISA/210.
    Cover Letter under 35 USC 371 and 1.495.
    Claim of Priority.

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APPLICATION NO. (If known, see 37 CFR	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUME
09/926718	PCT/FR00/01302	P21722

19. ___ The following fees are submitted:	CALCULATIONS	PTO USE C
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Search report has been prepared by the EPO or JPO. . . . . $ 890.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482). . . . . $ 710.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO(37 CFR 1.445(a)(2). . . . . $ 740.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO. $1,040.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4). . . . . $ 100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$890.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ___ 20 ___ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	RATE		
Total Claims	- 20 =		X $18.00	$0.00	
Independent Claims	- 3 =		X $84.00	$0.00	
Multiple dependent claim(s) (if applicable)			+ $280.00	$0.00	
			TOTAL OF ABOVE CALCULATIONS =	$890.00	
___ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by ½.				$	
			SUBTOTAL =	$890.00	
Processing fee of $130.00 for furnishing the English translation later than ___ 20 ___ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).			+		
Extension of Time fee in the amount of $					
			TOTAL NATIONAL FEE =	$890.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property			+		
			TOTAL FEES ENCLOSED =	$890.00	
			Amount to be refunded	$	
			Charged	$	

a. _X_ A check in the amount of $890.00 to cover the above fees is enclosed.

b. ___ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees.

c. _X_ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0089.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO **CUSTOMER NO. 7055**
AT THE PRESENT ADDRESS OF:
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07055
PATENT TRADEMARK OFFICE

Reg. No. 45,294
SIGNATURE
Neil F. Greenblum
NAME

28,394
REGISTRATION NUMBER

P21772.A03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : J. POINT et al.

Appl. No : 09/926,718
(U.S. National Phase of PCT/FR00/01302)
I.A. Filed : May 15, 2000

For : METHOD FOR OBTAINING FROM A CULTURE MEDIUM OF MICROALGAE, A HEAT-STABLE EXTRACT WITH ANTIOXIDANT AND WOUND HEALING ACTIVITY

PRELIMINARY AMENDMENT AND COVER LETTER SUBMITTING MODIFIED PAGES

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Applicants note that the Notification of Missing Requirements mailed January 25, 2002 acknowledges receipt of the International Preliminary Examination Report, and Applications are submitting herewith a copy of the International Preliminary Examination Report and the Annex in the French language. The International Preliminary Examination Report is drawn on description pages 1 - 6, and claims 1 - 15 (on pages 7 and 8) which is attached to the International Preliminary Examination Report as an Annex..

Moreover, Applicants submit herewith an English translation of the Annex, which is identified as "MODIFIED SHEET" in the lower margin.

Accordingly, Applicants requests examination of the Modified Sheets of application, including pages of description 1-5, and claims 1-15 (pages 6 and 7). Additionally, prior to the examination of the above-identified application, Applicants requests amendment of the claims to remove multiple dependent claims:

IN THE CLAIMS

Please amend claims 5, 8, 9, 11 and 12, as follows *(Marked-up copies of the amended claims are attached as an Appendix)*:

5. (Amended) Extract with antioxidant and wound-healing properties obtained according to the method which is the object of claim 1, characterized in that it contains at least 30 U/ml of superoxide dismutases like (SOD like) and at least 1 mg/ml of sulphated polysaccharides.

8. (Amended) Extract according to claim 6, characterized in that the separation is obtained by means of a cellulosic membrane with pore dimensions comprised between 1,000 and 50,000 daltons.

9. (Amended) Extract according to claim 6, characterized in that the precipitation is obtained by means of ethanol.

11. (Amended) Use of the extract according to claim 5, in the preparation of dietetic compositions against oxidative stress.

12. (Amended) Use of the extract according to claim 5, for the preservation of food products.

REMARKS

The Examiner is respectfully requested to enter the foregoing modified sheets and to amend the claims as noted above prior to examination and calculation of the filing fees in the above-identified patent application.

P21772.A03

Should there be any questions, the Examiner is invited to contact the undersigned at the below listed number.

Respectfully submitted,
J. POINT et al.

Neil P. Greenblum
Reg. No. 28,394

March 25, 2002
GREENBLUM & BERNSTEIN, P.L.C.
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APPENDIX

Marked-Up Copies of Amended Claims:

5. (Amended) Extract with antioxidant and wound-healing properties obtained according to the method which is the object of [claims 1-4] claim 1, characterized in that it contains at least 30 U/ml of superoxide dismutases like (SOD like) and at least 1 mg/ml of sulphated polysaccharides.

8. (Amended) Extract according to claim 6 [or claim 7], characterized in that the separation is obtained by means of a cellulosic membrane with pore dimensions comprised between 1,000 and 50,000 daltons.

9. (Amended) Extract according to claim 6 [or claim 7], characterized in that the precipitation is obtained by means of ethanol.

11. (Amended) Use of the extract according to claim 5 [or claim 6], in the preparation of dietetic compositions against oxidative stress.

12. (Amended) Use of the extract according to claim 5 [or claim 6], for the preservation of food products.

METHOD FOR OBTAINING FROM A CULTURE MEDIUM OF MICROALGAE, A HEAT-STABLE EXTRACT WITH ANTIOXIDANT AND WOUND HEALING ACTIVITY

[0001] The present invention relates to a method of obtaining, from a culture medium of microalgae, a heat-stable extract having an antioxidant and wound-healing activity associated with a high content of superoxide dismutases like and sulphated polysaccharides, and capable of finding applications in the chemical industry, cosmetic industry, pharmaceutical industry, and agronomic industry, as well as in the fields of nutraceutics and dietetics.

[0002] Numerous works have already been done, in the cosmetic filed, to develop antiradical substances capable of slowing skin aging. Concurrently, researches have been conducted in the medical sector to obtain products with anti-inflammatory activity, adapted to be used in particular in rheumatology and for the treatment of pathologies associated with oxidative stress and affecting the digestive and cardiovascular systems.

[0003] Over the past few years, the researches undertaken have been deliberately oriented toward the plant sector in order to avoid the infectious substances that may be found in animal extracts. Among these researches, the culture of microalgae has led to the obtaining of interesting products from the algal biomass.

[0004] The Patent No. EP 0 437 393 discloses a method and a photobioreactor adapted to the production and extraction of antioxidants from a culture of microorganisms, the method consisting of culturing in the photobioreactor microalgae suspended in a culture medium, the oxygen produced by the microalgae by photosynthesis being collected and then reinjected into the culture medium, separating the microalgae from the culture medium, dissolving them, grinding the solution, adding a solvent for solubilizing the antioxidants, and separating the liquid phases present.

[0005] The Patent No. EP 0 628 629 describes a process for the production and extraction of thermostable superoxide dismutases from a culture of microorganisms suspended in a culture medium and selected from among microalgae and cyanobacteria, this process consisting of culturing in a photoreactor aerobic, photosynthetic thermophilic microorganisms producing oxygen, and extracting the superoxide dismutases from the culture medium by cellular crushing, ultrafiltration and selective precipitation.

[0006] However, all of the techniques used have the disadvantages of being complex and

of resulting in the rejection of the culture medium of said microalgae.

[0007] The present invention is based on the discovery of unexpected properties of the culture medium of the microalgae which, under certain conditions, can produce large quantities of superoxide dismutases like (SOD like) and sulphated polysaccharides (SP).

[0008] Superoxide dismutases like (SOD) are products that have the same type of activity as superoxide dismutases (SOD), with the advantage over the latter of being heat-stable, which is not the case of enzymes in general, and of superoxide dismutases in particular.

[0009] Thus, the object of the present invention is a method of obtaining an extract from the culture medium of microalgae, this method being essentially characterized in that it consists of first culturing said microalgae, then subjecting them to oxygen supersaturation which produces a metabolic forcing leading to an overproduction of antioxidant compounds.

[0010] The microalgae used in the method according to the invention are preferably selected from the group of Rhodophycae, especially Porphyridium cruentum (PC).

[0011] According to the invention, the culture of the selected microalgae is carried out in a conventional-type photobioreactor, in which solar energy or an appropriate lighting makes it possible to perform photosynthesis based on said culture, under controlled conditions of temperature, pH, and the supply of carbon dioxide CO_2.

[0012] The supply of CO_2 for the culture medium, as well as the agitation necessary for a good development of the microalgae, can be ensured by a bubbling of air enriched with compressed CO_2 or by any other equivalent means.

[0013] After a sufficiently long culturing time, on the order of 6-12 days, when the cellular density of the culture medium is optimum, 100 mg/l of potassium bicarbonate are added thereto, then the reactor is closed and the culture is allowed to continue for a period of time on the order of 1-3 days, in order to create a metabolic forcing caused by the oxygen supersaturation of the medium coming from the photosynthesis, the potassium bicarbonate providing the carbon required by the photosynthesis.

[0014] Next, the algae are separated from the culture medium by centrifuging or filtering. A filtering by means of an appropriate filter then makes it possible to separate the antioxidant extract.

[0015] The filter used for separating the desired extract can be a cellulosic membrane with pore dimensions of 1-1.5µm.

[0016] The product thus extracted from the culture medium of the microalgae contains at least 30 U/ml of SOD like, this measurement being performed by means of the kit sold

under the name "SOD-525." It further contains at least 1 mg/ml of sulphated polysaccharides.

[0017] The high content of SOD like in the extract according to the invention provides it with anti-inflammatory properties that can find an application in rheumatology, as well as antiradical properties that can be used in cosmetics, in the preparation of cream against the aging of skin.

[0018] Moreover, the high content of sulphated polysaccharides in the extract according to the invention provides it with tissue regeneration properties which, associated with the SOD-like antiradical properties, make it an excellent wound-healing product.

[0019] The product according to the invention can also be used to reinforce the antioxidant activities of other plant extracts, in particular for the biological control of plant parasites. Thus, products with peroxidasic activity can benefit from a markedly increased activity by adding about 10% of the extract according to the invention.

[0020] The product according to the invention has the advantage of being heat-stable, especially at 121°C for 20 minutes.

[0021] According to the intended application for the extract according to the invention, it can be used as obtained according to the method described hereinabove. However, the active compounds can also be separated so as to obtain two products with different activities, one containing the SOD-like, the other the sulphated polysaccharides.

[0022] The separation of the portion containing the SOD-like can be carried out by precipitation by means of a solvent such as ethanol, or by separation by means of an organic membrane such as a cellulose membrane, with pore dimensions comprised between 1,000 and 50,000 daltons. Two extracts are obtained, of which, containing the SOD-like, has an antioxidant and antiradical activity, whereas the other, containing the sulphated polysaccharides, has a tissue regeneration activity.

[0023] The present invention will be better understood by means of the examples that follow, which are provided by way of a mere illustration of the invention, with respect to which they are in no way limiting.

EXAMPLE 1

Obtaining of an extract from the culture medium of Porphyridium cruentum (PC)

[0024] In a 150 liter photobioreactor, the alga is cultured in a Conway-type medium - artificial sea water. The inoculation is done by 5 l of culture at 5 million cells per milliliter. An air bubbling is ensured by solar energy or by lamps distributing 100 µ moles/m2/s.

[0025] After 12 days, when the cellular density is optimum, 100 mg/liter, or 15 g of KHCO3 is added; then the reactor is closed and the culture is allowed to continue for two days in order to create a metabolic forcing.

[0026] After that time, the algae are separated from the culture medium by centrifuging, next the latter is filtered on a cellulose membrane having pores of 1.2 μm

[0027] The extract thus obtained has a SOD like content of 30 U/ml, measured with the SOD-525 kit, and a sulphated polysaccharides content of 1 mg/ml.

[0028] This extract can be sterilized at 121°C for 20 minutes, then packaged in sterile flasks, without this operation modifying the SOD activity, nor the SP content.

[0029] It can also be dehydrated by lyophilization and then be used in the preparation of compositions such as pills.

EXAMPLE 2

Separation of the activities

[0030] From 10 liters of the extract obtained in Example 1, the SOD like portion is separated from the sulphated polysaccharides by ethanol treatment, which precipitate.

[0031] Two extracts are thus obtained, one of which has an antiradical activity unchanged by the separation, and the other of which contains the sulphated polysaccharides and has a tissue regeneration activity.

[0032] Each of the two extracts can be preserved in an aqueous form, after sterilization and packaging in sterile flasks.

[0033] The extracts can also be preserved in soluble powder form, after lyophilization.

EXAMPLE 3

COSMETIC PRODUCTS

1) Cream against the aging of skin

[0034] In an appropriate mixer, the following mixture, expressed in percentage by weight, is obtained:

EXTRACT according to Example 1	10
PEG -8 BEESWAX	8
OCTYDODECYL MYRISTATE	10
ISOSTEARYL ISOSTERARATE	10
SODIUM HYDROXIDE	0.4
ETHOXYDIGLYCOL	5
WATER, PERFUME, PRESERVATIVE	QSP 100

[0035] An in vitro objectivation of the cream thus obtained makes it possible to determine a fibroblast proliferation, even in the presence of anti-proliferative agents introduced into the medium, which constitutes the indicator of a trophic and reconstituting effect.

2) Brumisateur softening spray for delicate skin

[0036] The liquid of Example 1 is packaged in a nitrogen spraying flask.

EXAMPLE 4

Dietetic composition against oxidative stress

[0037] By means of the extract obtained in Example 1, 250 mg powder capsules are obtained, intended for nutraceutics and parapharmacy.

[0038] Tests performed on rats, at a daily dose of 5 mg of powder, have shown an increase in antiradical substances in blood plasma.

EXAMPLE 5

Anti-inflammatory eyewash

[0039] An eyewash is prepared according to the following formula:

Chlorhexidine gluconate	0.005 g
Dehydrated inosine disodium phosphate	0.5 g
Liquid extract according to Example 1 qsq	100 ml

[0040] The eyewash thus obtained can be used for treating pink eye.

EXAMPLE 6

Food product

[0041] 0.1% by weight of the powder of Example 1 is added to a 50 degree Brix tomato concentrate in order to increase its shelf life.

EXAMPLE 7

Composition against plant parasites

[0042] The extract containing the SP of Example 2 applied to vegetables such as melon or vine induces a peroxidasic activity which is the sign of a potential resistance to parasites such as mushrooms.

EXAMPLES 8

Application to polymers

[0043] The extract obtained in example 1 can be incorporated into polymers, after dehydration, to increase the resistance to oxidation.

CLAIMS

1. Method of obtaining, from the culture medium of microalgae, a heat-stable extract having an antioxidant and wound-healing activity, consisting of first culturing said microalgae in a photobioreactor subject to an appropriate lighting and to controlled conditions of temperature, pH, and the supply of carbon dioxide CO_2, then subjecting them to oxygen supersaturation, then separating the algae from the culture medium by centrifuging, and finally filtering said culture medium on an appropriate filter for separating said extract,

characterized in that after a period of six to twelve days, 100 mg/l of potassium bicarbonate are added to the culture medium, then the reactor is closed for a period of one to three days to obtain the oxygen supersaturation.

2. Method according to claim 1, characterized in that the lighting of the photobioreactor is obtained by solar energy.

3. Method according to claim 1, characterized in that the supply of CO_2 is carried out by bubbling of air enriched with compressed CO_2.

4. Method according to claim 1, characterized in that the filtering of the culture medium after the centrifuging, adapted to separate the algae therefrom, is carried out on a filter constituted of cellulosic membrane with pore dimensions comprised between 1 and 1.5 µm.

5. Extract with antioxidant and wound-healing properties obtained according to the method which is the object of claims 1-4, characterized in that it contains at least 30 U/ml of superoxide dismutases like (SOD like) and at least 1 mg/ml of sulphated polysaccharides.

6. Extract with antiradical properties obtained from the extract according to claim 5 by precipitation by means of a solvent or by separation by means of a cellulosic membrane, characterized in that it contains at least 30 U/ml of SOD like.

7. Extract with tissue regeneration properties obtained from the extract according to claim 5, by precipitation by means of a solvent or by separation by means of a cellulosic membrane, characterized in that it contains at least 1 mg/ml of sulphated polysaccharides.

8. Extract according to claim 6 or claim 7, characterized in that the separation is obtained by means of a cellulosic membrane with pore dimensions comprised between 1,000 and 50,000 daltons.

9. Extract according to claim 6 or claim 7, characterized in that the precipitation is

obtained by means of ethanol.

10. Use of the extract according to claim 5, as an antioxidant in the manufacture of polymers.

11. Use of the extract according to claim 5 or claim 6, in the preparation of dietetic compositions against oxidative stress.

12. Use of the extract according to claim 5 or claim 6, for the preservation of food products.

13. Use of the extract according to claim 5 in the preparation of cosmetic products products adapted to slow skin aging.

14. Use of the extract according to claim 5 in the preparation of anti-inflammatory compositions, especially in rheumatology.

15. Use of the extract according to claim 7 in the preparation of compositions adapted to the biological control of plant parasites.

METHOD FOR OBTAINING FROM A CULTURE MEDIUM OF MICROALGAE, A HEAT-STABLE EXTRACT WITH ANTIOXIDANT AND WOUND HEALING ACTIVITY

[0001] The present invention relates to a method of obtaining, from a culture medium of microalgae, a heat-stable extract having an antioxidant and wound-healing activity associated with a high content of superoxide dismutases like and sulphated polysaccharides, and capable of finding applications in the chemical industry, cosmetic industry, pharmaceutical industry, and agronomic industry, as well as in the fields of nutraceutics and dietetics.

[0002] Numerous works have already been done, in the cosmetic filed, to develop antiradical substances capable of slowing skin aging. Concurrently, researches have been conducted in the medical sector to obtain products with anti-inflammatory activity, adapted to be used in particular in rheumatology and for the treatment of pathologies associated with oxidative stress and affecting the digestive and cardiovascular systems.

[0003] Over the past few years, the researches undertaken have been deliberately oriented toward the plant sector in order to avoid the infectious substances that may be found in animal extracts.

[0004] Among these researches, the culture of microalgae has led to the obtaining of interesting products from the algal biomass. However, the techniques used have the disadvantage of being complex and of resulting in the rejection of the culture medium of said microalgae.

[0005] The present invention is based on the discovery of unexpected properties of the culture medium of the microalgae which, under certain conditions, can produce large quantities of superoxide dismutases like (SOD like) and sulphated polysaccharides (SP).

[0006] Superoxide dismutases like (SOD) are products that have the same type of activity as superoxide dismutases (SOD), with the advantage over the latter of being heat-stable, which is not the case of enzymes in general, and of superoxide dismutases in particular.

[0007] Thus, the object of the present invention is a method of obtaining an extract from the culture medium of microalgae, this method being essentially characterized in that it consists of first culturing said microalgae, then subjecting them to oxygen supersaturation which produces a metabolic forcing leading to an overproduction of antioxidant compounds.

[0008] The microalgae used in the method according to the invention are preferably selected from the group of Rhodophycae, especially Porphyridium cruentum (PC).

[0009] According to the invention, the culture of the selected microalgae is carried out in a conventional-type photobioreactor, in which solar energy or an appropriate lighting makes it possible to perform photosynthesis based on said culture, under controlled conditions of temperature, pH, and the supply of carbon dioxide CO^2.

[0010] The supply of CO^2 for the culture medium, as well as the agitation necessary for a good development of the microalgae, can be ensured by a bubbling of air enriched with compressed CO^2 or by any other equivalent means.

[0011] After a sufficiently long culturing time, on the order of 6-12 days, when the cellular density of the culture medium is optimum, 100 mg/l of potassium bicarbonate are added thereto, then the reactor is closed and the culture is allowed to continue for a period of time on the order of 1-3 days, in order to create a metabolic forcing caused by the oxygen supersaturation of the medium coming from the photosynthesis, the potassium bicarbonate providing the carbon required by the photosynthesis.

[0012] Next, the algae are separated from the culture medium by centrifuging or filtering. A filtering by means of an appropriate filter then makes it possible to separate the antioxidant extract.

[0013] The filter used for separating the desired extract can be a cellulosic membrane with pore dimensions of 1-1.5µm.

[0014] The product thus extracted from the culture medium of the microalgae contains at least 30 U/ml of SOD like, this measurement being performed by means of the kit sold under the name "SOD-525." It further contains at least 1 mg/ml of sulphated polysaccharides.

[0015] The high content of SOD like in the extract according to the invention provides it with anti-inflammatory properties that can find an application in rheumatology, as well as antiradical properties that can be used in cosmetics, in the preparation of cream against the aging of skin.

[0016] Moreover, the high content of sulphated polysaccharides in the extract according to the invention provides it with tissue regeneration properties which, associated with the SOD-like antiradical properties, make it an excellent wound-healing product.

[0017] The product according to the invention can also be used to reinforce the antioxidant activities of other plant extracts, in particular for the biological control of plant parasites. Thus, products with peroxidasic activity can benefit from a markedly increased activity by adding about 10% of the extract according to the invention.

[0018] The product according to the invention has the advantage of being heat-stable,

especially at 121°C for 20 minutes.

[0019] According to the intended application for the extract according to the invention, it can be used as obtained according to the method described hereinabove. However, the active compounds can also be separated so as to obtain two products with different activities, one containing the SOD-like, the other the sulphated polysaccharides.

[0020] The separation of the portion containing the SOD-like can be carried out by precipitation by means of a solvent such as ethanol, or by separation by means of an organic membrane such as a cellulose membrane, with pore dimensions comprised between 1,000 and 50,000 daltons. Two extracts are obtained, of which, containing the SOD-like, has an antioxidant and antiradical activity, whereas the other, containing the sulphated polysaccharides, has a tissue regeneration activity.

[0021] The present invention will be better understood by means of the examples that follow, which are provided by way of a mere illustration of the invention, with respect to which they are in no way limiting.

EXAMPLE 1

Obtaining of an extract from the culture medium of Porphyridium cruentum (PC)

[0022] In a 150 liter photobioreactor, the alga is cultured in a Conway-type medium - artificial sea water. The inoculation is done by 5 l of culture at 5 million cells per milliliter. An air bubbling is ensured by solar energy or by lamps distributing 100 μ moles/m^2/s.

[0023] After 12 days, when the cellular density is optimum, 100 mg/liter, or 15 g of $KHCO_3$ is added; then the reactor is closed and the culture is allowed to continue for two days in order to create a metabolic forcing.

[0024] After that time, the algae are separated from the culture medium by centrifuging, next the latter is filtered on a cellulose membrane having pores of 1.2 μm

[0025] The extract thus obtained has a SOD like content of 30 U/ml, measured with the SOD-525 kit, and a sulphated polysaccharides content of 1 mg/ml.

[0026] This extract can be sterilized at 121°C for 20 minutes, then packaged in sterile flasks, without this operation modifying the SOD activity, nor the SP content.

[0027] It can also be dehydrated by lyophilization and then be used in the preparation of compositions such as pills.

EXAMPLE 2

Separation of the activities

[0028] From 10 liters of the extract obtained in Example 1, the SOD like portion is separated from the sulphated polysaccharides by ethanol treatment, which precipitate.

[0029] Two extracts are thus obtained, one of which has an antiradical activity unchanged by the separation, and the other of which contains the sulphated polysaccharides and has a tissue regeneration activity.

[0030] Each of the two extracts can be preserved in an aqueous form, after sterilization and packaging in sterile flasks.

[0031] The extracts can also be preserved in soluble powder form, after lyophilization.

EXAMPLE 3

COSMETIC PRODUCTS

1) Cream against the aging of skin

[0032] In an appropriate mixer, the following mixture, expressed in percentage by weight, is obtained:

EXTRACT according to Example 1	10
PEG -8 BEESWAX	8
OCTYDODECYL MYRISTATE	10
ISOSTEARYL ISOSTERARATE	10
SODIUM HYDROXIDE	0.4
ETHOXYDIGLYCOL	5
WATER, PERFUME, PRESERVATIVE	QSP 100

[0033] An in vitro objectivation of the cream thus obtained makes it possible to determine a fibroblast proliferation, even in the presence of anti-proliferative agents introduced into the medium, which constitutes the indicator of a trophic and reconstituting effect.

2) Brumisateur softening spray for delicate skin

[0034] The liquid of Example 1 is packaged in a nitrogen spraying flask.

EXAMPLE 4

Dietetic composition against oxidative stress

[0035] By means of the extract obtained in Example 1, 250 mg powder capsules are obtained, intended for nutraceutics and parapharmacy.

[0036] Tests performed on rats, at a daily dose of 5 mg of powder, have shown an increase in antiradical substances in blood plasma.

EXAMPLE 5

Anti-inflammatory eyewash

[0037] An eyewash is prepared according to the following formula:

Chlorhexidine gluconate	0.005 g

Dehydrated inosine disodium phosphate	0.5 g
Liquid extract according to Example 1 qsq	100 ml

[0038] The eyewash thus obtained can be used for treating pink eye.

EXAMPLE 6

Food product

[0039] 0.1% by weight of the powder of Example 1 is added to a 50 degree Brix tomato concentrate in order to increase its shelf life.

EXAMPLE 7

Composition against plant parasites

[0040] The extract containing the SP of Example 2 applied to vegetables such as melon or vine induces a peroxidasic activity which is the sign of a potential resistance to parasites such as mushrooms.

EXAMPLE 8

Application to polymers

[0041] The extract obtained in example 1 can be incorporated into polymers, after dehydration, to increase the resistance to oxidation.

CLAIMS

1. Method of obtaining, from the culture medium of microalgae, a heat-stable extract having an antioxidant and wound-healing activity, characterized in that it consists of first culturing said microalgae, subjecting them to oxygen supersaturation, then separating the algae from the culture medium by centrifuging, and finally filtering said culture medium on an appropriate filter for separating said extract.

2. Method according to claim 1, characterized in that the culturing of the microalgae is carried out in a photobioreactor subject to an appropriate lighting, under controlled conditions of temperature, pH, and the supply of carbon dioxide CO^2.

3. Method according to claim 2, characterized in that the lighting of the photobioreactor is obtained by solar energy or by an appropriate lighting.

4. Method according to claim 2, characterized in that the supply of CO^2 is carried out by bubbling of air enriched with compressed CO^2.

5. Method according to claim 1, characterized in that after a period of 10 to 12 days, 100 mg/l of potassium bicarbonate are added to the culture medium, then the reactor is closed for a period of 1 to 3 days to obtain the oxygen supersaturation.

6. Method according to claim 1, characterized in that the filtering of the culture medium after the centrifuging, adapted to separate the algae therefrom, is carried out on a filter constituted of cellulosic membrane with pore dimensions comprised between 1 and 1.5 µm.

7. Extract with antioxidant and wound-healing properties obtained according to the method which is the object of claims 1-6, characterized in that it contains at least 30 U/ml of superoxide dismutases like (SOD like) and at least 1 mg/ml of sulphated polysaccharides.

8. Extract with antiradical properties obtained from the extract according to claim 7 by precipitation by means of a solvent or by separation by means of a cellulosic membrane, characterized in that it contains at least 30 U/ml of SOD like.

9. Extract with tissue regeneration properties obtained from the extract according to claim 7, by precipitation by means of a solvent or by separation by means of a cellulosic membrane, characterized in that it contains at least 1 mg/ml of sulphated polysaccharides.

10. Extract according to claim 8 or claim 9, characterized in that the separation is obtained by means of a cellulosic membrane with pore dimensions comprised between 1,000 and 50,000 daltons.

11. Extract according to claim 8 or claim 9, characterized in that the precipitation

is obtained by means of ethanol.

12. Use of the extract according to claim 7, as an antioxidant in the manufacture of polymers.

13. Use of the extract according to claim 7 or claim 8, in the preparation of dietetic compositions against oxidative stress.

14. Use of the extract according to claim 7 or claim 8, for the preservation of food products.

15. Use of the extract according to claim 7 in the preparation of cosmetic products adapted to slow skin aging.

16. Use of the extract according to claim 7 in the preparation of anti-inflammatory compositions, especially in rheumatology.

17. Use of the extract according to claim 9 in the preparation of compositions adapted to the biological control of plant parasites.

Declaration and Power of Attorney For Utility or Design Patent Application

Déclaration pour Demandes de Brevet d'Utilité et de Modèle avec Pouvoirs

French Language Declaration

En tant qu'inventeur nommé ci-après, Je déclare par le présent acte que:

Mon domicile, mon adresse postale et ma nationalité sont ceux figurant ci-dessous à côté de mon nom.

Je crois être le premier inventeur original et unique (si un seul nom est mentionné ci-dessous), ou l'un des premiers co-inventeurs originaux (si plusieurs noms sont mentionnés ci-dessous) de l'objet revendiqué, pour lequel une demande de brevet a été déposée concernant l'invention intitulée

PROCEDE D'OBTENTION, A PARTIR DU MILIEU DE CULTURE DE MICRO ALGUES, D'UN EXTRAIT THERMOSTABLE POSSEDANT UNE ACTIVITE ANTI-OXYDANTE ET PRO-CICATRISANTE

et dont la description est fournie ci-jointe à moins que la case suivante n'ait été cochée:

■ a été déposée 15 mai 2000
sous le numéro de demande des Etats-Unis _____
et modifiée le _____ (le cas échéant)
ou,

☐ le numéro de demande internationale PCT PCT/FR00/01302
et modifiée le _____ (le cas échéant).

Je déclare par le présent acte avoir passé en revue et compris le contenu de la description ci-dessus, revendications comprises, telles que modifiées par toute modification dont il aura été fait référence ci-dessus.

Je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations.

Je revendique par le présent acte avoir la priorité étrangère, en vertu du Titre 35, §119(a)-(d) ou §365(b) du Code des Etats-Unis, sur toute demande étrangère de brevet ou certificat d'inventeur ou, en vertu du Titre 35, §365(a) du même Code, sur toute demande internationale PCT désignant au moins un pays autre que les Etats-Unis et figurant ci-dessous. J'ai aussi indiqué ci-dessous, en cochant la case "Non", toute demande étrangère de brevet, tout certificat d'inventeur ou toute demande internationale PCT ayant une date de dépôt précédant celle de la demande à propos de laquelle une priorité est revendiquée.

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

METHOD FOR OBTAINING FROM A CULTURE MEDIUM OF MICROALGAE, A HEAT-STABLE EXTRACT WITH ANTIOXIDANT AND WOUND HEALING ACTIVITY

the specification of which is attached hereto unless the following box is checked:

■ was filed on May 15, 2000 as
United States Application Number _____
and was amended on _____ (if applicable)
or,

PCT International Application Number PCT/FR00/01302
and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority under Title 35, United States Code §119 (a-d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States, listed below. I have also identified below, by checking the "No" box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed:

Prior foreign applications Demandes antérieures étrangères			Priority claimed Priorité revendiquée	
99/07372	France	8/Juni/1999	■	☐
(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Année de dépôt)	Yes Oui	No Non
			☐	☐
(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Année de dépôt)	Yes Oui	No Non

☐ D'autres demandes étrangères sont énumérées sur la feuille de priorité supplémentaire ci-jointe.

☐ Additional foreign application numbers are listed on a supplemental priority sheet attached hereto.

French Language Utility or Design Patent Application Declaration

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35 §119(e) du Code des Etats-Unis, de toute demande de brevet provisoire effectuée aux Etats-Unis et figurant ci-dessous.

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

(Application No.) (No. de la demande)	(Day/Month/Year Filed) (Jour/Mois/Année de dépôt)
(Application No.) (No. de la demande)	(Day/Month/Year Filed) (Jour/Mois/Année de dépôt)
(Application No.) (No. de la demande)	(Day/Month/Year Filed) (Jour/Mois/Année de dépôt)

☐ D'autres demandes provisoires sont énumérées sur la feuille de priorité supplémentaire ci-jointe.

☐ Additional provisional application numbers are listed on a supplemental priority sheet attached hereto.

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, §120 du Code des Etats-Unis, de toute demande de brevet effectuée aux Etats-Unis, ou en vertu du Titre 35, §365 (c) du même Code, de toute demande internationale PCT désignant les Etats-Unis et figurant ci-dessous et, dans la mesure où l'objet de chacune des revendications de cette demande de brevet n'est pas divulgué dans la demande antérieure américaine ou internationale PCT, en vertu des dispositions du premier paragraphe du Titre 35, §112 du Code des Etats-Unis, je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, §1.56 du Code fédéral des réglementations, dont j'ai pu disposer entre la date de dépôt de la demande antérieure et la date de dépôt de la demande nationale ou internationale PCT de la présente demande:

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s), or §365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application No.) (No. de la demande)	(Day/Month/Year Filed) (Jour/Mois/Année de dépôt)	(Status) (Etat) (patented, pending, abandoned) (brevetée, pendante, abandonnée)
(Application No.) (No. de la demande)	(Day/Month/Year Filed) (Jour/Mois/Année de dépôt)	(Status) (Etat) (patented, pending, abandoned) (brevetée, pendante, abandonnée)

☐ D'autres demandes américaines ou internationales sont énumérées sur la feuille de priorité supplémentaire ci-jointe.

☐ Additional U.S. or international application numbers are listed on a supplemental priority sheet attached hereto.

Je déclare par le présent acte que toute déclaration ci-incluse est, à ma connaissance, véridique et que toute déclaration formulée à partir de renseignements ou de suppositions est tenue pour véridique; et de plus, que toutes ces déclarations ont été formulées en sachant que toute fausse déclaration volontaire ou son équivalent est passible d'une amende ou d'une incarcération, ou des deux, en vertu de la Section 1001 du Titre 18 du Code des Etats-Unis, et que de telles déclarations volontairement fausses risquent de compromettre la validité de la demande de brevet ou du brevet délivré à partir de celle-ci.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Le(s) soussigné(s) autorise(nt) par la présente le(s) avocat(s) américain(s) ou le(s) mandataire(s) ci-après désigné(s) à accepter et à suivre les instructions, soit de son(leurs) conseil(s) en brevet étranger(s), soit du représentant officiel de la société, concernant toute démarche nécessaire à effectuer auprès de l'Office américain des Brevets et des Marques concernant cette demande, sans communication directe entre le(s) avocat(s) américain(s) ou le(s) mandataire(s) nommé(s) par la présente sera(ont) informé(s) par le(s) soussigné(s). Dans l'hypothèse d'un changement dans les donneurs d'instructions, le(s) avocat(s) américain(s) ou le(s) mandataire(s) nommé(s) par la présente sera(ont) informé(s) par le(s) soussigné(s).

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from either his foreign patent agent or corporate representative, if any, as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

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POUVOIR: En tant qu'inventeur, je désigne l'(les) avocat(s) et/ou l'(les) agent(s) associés au Numéro Client indiqué ci-dessous pour poursuivre la procédure de cette demande et traiter toute affaire la concernant auprès de l'Office des Brevets et des Marques, et autorise à ce que toute correspondance soit associée à ce Numéro Client.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the attorney(s) and/or agent(s) associated with the Customer Number provided below to prosecute this application and transact all business in the Patent and Trademark Office connected therewith, and direct that all correspondence be addressed to that Customer Number:

NUMERO CLIENT 7055

CUSTOMER NUMBER 7055

Les avocats actuellement désignés sont énumérés ci-après:

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(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire).	(Supply similar information and signature for third and subsequent joint inventors.)

French Language Utility or Design Patent Application Declaration

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Nationalité	Citizenship
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Nom complet du sixième co-inventeur, le cas échéant	Full name of sixth inventor, if any
Signature du sixième inventeur Date	Sixth Inventor's signature Date
Domicile	Residence
Nationalité	Citizenship
Adresse Postale	Post Office Address
(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire).	(Supply similar information and signature for seventh and subsequent joint inventors).